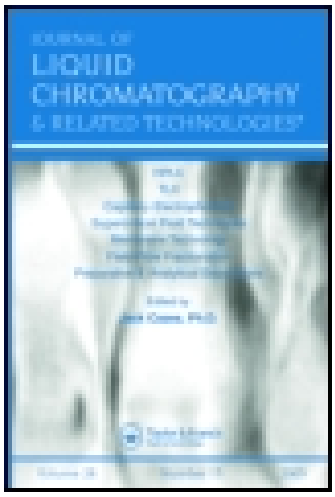

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C_{18}-Functionalized Magnetic Silica Nanoparticles for Solid Phase Extraction of Microcystin-LR in Reservoir Water Samples Followed by HPLC-DAD Determination

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C_{18}-FUNCTIONALIZED MAGNETIC SILICA NANOPARTICLES FOR SOLID PHASE EXTRACTION OF MICROCYSTIN-LR IN RESERVOIR WATER SAMPLES FOLLOWED BY HPLC-DAD DETERMINATION

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Abstract

In this study, C_{18}-functionalized magnetic silica nanoparticles (Fe_3O_4@SiO_2@C_{18} MNPs) based magnetic solid phase extraction (MSPE) was successfully developed for the determination of microcystin-LR (MC-LR) in reservoir water samples followed by high performance liquid chromatography-diode array detection (HPLC-DAD). After the extraction, the adsorbent can be conveniently and rapidly separated from aqueous samples by an external magnet. The main factors influencing the extraction efficiency including the amount of the MNPs, the extraction time, the pH of sample solution and desorption conditions were optimized to obtain high recoveries and extraction efficiency. High enrichment factor 500 was attained. Under the optimized experimental conditions, the calibration curve of MC-LR was linear in the range of 0.1–10.0 μg/L with the correlation coefficients (r^2) 0.9996. Limit of detection (LOD, S/N=3) of the method was 0.056 μg/L. The developed method was successfully applied to the determination of

MC-LR in reservoir water samples. The method recoveries were obtained ranging from 73.3–104% for three spiked concentrations, with the relative standard deviations (RSD) of 2.90–4.30%. The developed Fe_3O_4@SiO_2@C_{18} MNPs based MSPE coupled with HPLC-DAD demonstrated excellent sensitivity and repeatability, simplicity, rapidity and ease of operation, as well as practical applicability.

KEYWORDS: C_{18}-functionalized magnetic silica nanoparticles, HPLC-DAD, magnetic solid phase extraction, microcystins, water samples

INTRODUCTION

In recent years, cyanobacterial blooms occur frequently in eutrophic freshwater body. A variety of toxins can be released by algae cells rupture. Among them, microcystins (MCs) are the highest frequency, the largest quantity and the most serious harm type.[1] MCs are a family of hepatic toxins, which have been considered as potential tumor promoters. They are also responsible for the poisoning death of wild animals, livestock, poultry, *etc*.[2] MCs are a kind of monocylic heptapeptide with biological activity. Its general structure (Figure 1) is cyclo (D-Ala-X-D-MeAsp-Z-Adda-D-Glu-Mdha) and relative molecular weight is about 1000.[3] There are more than 60 MCs isoforms that have been identified.[4] MC-LR is one of the most frequent variants. Owing to the increased concerns about the public health risks associated with MCs intake, the WHO recommends a provisional level of 1 μg/L for MC-LR concentration in drinking water.[5]

To develop a simple, sensitive and rapid detection method for MCs is necessary and important. The most widespread analytical techniques for MCs include commercial enzyme-linked immunosorbent assays (ELISA),[6] liquid chromatography with UV detection (HPLC-UV),[7] capillary electrophoresis (CE)[8] and liquid chromatography–mass spectrometry (LC–MS).[9–11] LC–MS offers the advantages of providing specificity and good sensitivity, however, the expensive cost makes it difficultly popularize. HPLC is a powerful tool to separate microsystins, however, UV detector cannot provide the similar sensitivity and selectivity to LC–MS without enrichment prior to analysis, since the concentration of MCs in water is very low (usually at the level of ng/L–μg/L). Therefore, pre-treatment techniques are needed for the enrichment and clean-up of MCs in environmental samples, in order to achieve the ideal determination sensitivity and effectively eliminate contaminants from complex samples. Up to now, the reported pre-treatment techniques for MCs include solid-phase extraction (SPE),[12–16] solid-phase microextraction (SPME),[17] cloud point extraction (CPE)[18,19] and so on. Amongst them, SPE is typically utilized. An HPLC method with UV detection after SPE was published as an ISO 20179 international standard.[12] SPE is also listed as the National Standard Method of China for determination of MCs in water (GB/T 20466-2006). Compared with liquid-liquid extraction, SPE has higher enrichment efficiency, uses less organic solvents and doesn't produce emulsification phenomenon. This method is easy to realize automation.[20] However, the porous structure of the stationary phase is easily jamed by the complex samples containing colloid or solid

granules, resulting in the lower column capacity and extraction efficiency. Moreover, for large volume of water sample, the pretreatment process needs long time, typically 2–4 h for 1 L water sample. Compared with SPE method, SPME has the following advantages: without organic solvent, no need for clean-up procedures, simple operation and short analysis time.[21] However, SPME fibers are comparatively expensive and have a limited lifetime, as they tend to degrade with repeated usage.[22]

Recently, sample extraction by magnetic nanoparticles (MNPs) has increasing attention due to significantly higher surface area-to-volume ratio and superparamagnetic property.[23–27] The Fe_3O_4 nanoparticles adsorbed with target compounds can be easily collected by an external magnetic field outside the extraction container without additional centrifugation or filtration of the sample.[28] To avoid alteration of the magnetic properties of magnetite or its oxidation, Fe_3O_4 nanoparticles are often coated with silica. The silica coating was subsequently functionalized with organosilanes and/or affinity ligands in order to enable the selective extraction of organic contaminants. The Fe_3O_4@SiO_2 nanoparticles modified with alkyl C_{18} (Fe_3O_4@SiO_2@C_{18}) are mostly applied, such as for the determination of methylprednisolone in rat plasma,[29] ergosterol in cigarettes,[30] polycyclic aromatic hydrocarbons in aqueous samples[24] and organophosphorous pesticides in environmental water.[31]

Despite the high concentrating potential of nanomaterial, and the ease of handling MNPs, no MNPs-based SPE have been used to concentrate microcystins in water. The aim of this work was to develop a sensitive analytical method to determine MC-LR in environmental water samples. Laboratory-made $Fe_3O_4@SiO_2@C_{18}$ MNPs were utilized for SPE procedure followed by HPLC analysis. Several key influence factors including the amount of the MNPs, the extraction time, the pH of sample solution and desorption conditions were optimized to obtain high recoveries and extraction efficiency. The method was demonstrated to be applicable for the analysis of MC-LR in real reservoir water samples.

EXPERIMENTAL

Reagents And Materials

Methanol (HPLC grade) was purchased from Merck (Darmstadt, Germany). Trifuoroacetic acid (TFA) of HPLC grade was from Dima Tech. (USA). Water was purified to 18.2 MΩ on a Synergy 185 ultrapure water system (Millipore, USA). MC-LR (10 μg/mL) standard solution was from Institute of Hydrobiology, CAS (China), which was stored and refrigerated at –20 ºC. The working standard solution was freshly prepared by diluting the standard solution with ultrapure water to required concentrations. $FeCl_3 \cdot 6H_2O$, ethylene glycol, ammonium hydroxide, absolute ethyl alcohol and NaAc were analytical reagents. Polyethylene glycol (Alfa Aesar), trichloro (octadecyl) silane

(Alfa Aesar), TEOS, trimethylchorosilane and triethylamine (Alfa Aesar) were used. Toluene was HPLC grade.

Preparation Of C_{18}-Functionalized Magnetic Silica Nanoparticles

C_{18}-functionalized magnetic silica nanoparticles (Fe_3O_4@SiO_2@C_{18} MNPs) were synthesized according to the previously reported method.[24] The route for preparation of Fe_3O_4@SiO_2@C_{18} MNPs was illustrated in Figure 2. Firstly, the magnetic Fe_3O_4 microspheres were synthesized by a solvothermal reduction method; secondly, the Fe_3O_4 microspheres were modified with TEOS; thirdly, C_{18} chain was bonded to the surface of silica gel modified magnetic microspheres through the Si–O–Si combination.

Apparatus And Measurement

The characterizations of magnetic silica NPs and C_{18}-functionalized magnetic silica nanoparticles were conducted on a Tescan XM 5136 scanning electron microscope (SEM, Tescan, Czech Republic) and Fourier-transform infrared spectrometry (FT-IR, Frontier, Perkin Elmer, USA).

Experiments were performed on an Agilent 1100 liquid chromatographic system, consisting of a quaternary delivery pump, an auto-sampler with a 100 μL loop, a thermostated column compartment and a DAD detector. A personal computer equipped with an Agilent ChemStation program for HPLC was used to process the

chromatographic data. The analytical column was Agela Vensuil MP-C_{18} (250 × 4.6 mm i.d., 0.5 μm), which was used for analysis of MC-LR at room temperature. The sample injection volume was 20 μL. The absorbance was monitored at 238 nm. The mobile phase was methanol-water (0.05% TFA) (60:40, v/v). The flow rate was 1 mL/min.

Magnetic Solid Phase Extraction (Mspe) Procedures

30 mg magnetic C_{18} microspheres were put into a 2 L beaker and firstly cleaned and activated with 5mL methanol and 10 mL distilled water in sequence. The beaker was placed in an ultrasound bath for 1min. Then 1 L of MC-LR aqueous solution was added into the beaker. The mixture was sonicated at room temperature for 7 min to form a homogeneous dispersion solution. Then magnetic C_{18} microspheres adsorbed MC-LR were separated rapidly from the solution under a strong external magnetic field. After discarding the supernatant solution, MC-LR was eluted from the magnetic C_{18} microspheres with 2 × 5 mL of methanol sonicated at room temperature for 3 min. The effluents were collected into a test tube and condensed to dryness under a gentle flow of nitrogen at room temperature and re-dissolved with 0.2 mL methanol. The resulting solution was then transferred to double layer silicone-teflon sept vials for autosampler and analysis by HPLC-DAD. The MSPE process was schematically shown in Figure 2.

RESULTS AND DISCUSSION

Characterization Of The C_{18}-Functionalized Magnetic Silica Nanoparticles

The magnetic C_{18} microspheres were strong enough to be easily separated by external magnetic field, as seen from Figure 3. The size and shape of the prepared microspheres were examined by SEM. As observed, the prepared magnetic C_{18} microspheres are homogeneous and spherical, having uniform sizes in the range of 100–190 nm (Figure 3).

FT-IR spectroscopy was applied to characterize the magnetic silica microspheres before and after modification with silane coupling agent. Figure 4 shows the FT-IR spectra of Fe_3O_4, $Fe_3O_4@SiO_2$ and $Fe_3O_4@SiO_2@C_{18}$. The absorption peak at 580 cm^{-1} is from Fe–O–Fe vibration of magnetite, and 1080 cm^{-1} is attributed to the Si–O–Si stretching vibration of silica layer formed on the surface of magnetite particles. After surface modification, the new emergence of absorption peaks at 2921 cm^{-1} and 2853 cm^{-1} is ascribed to C–H originated from silane coupling agent, suggesting the alkyl groups have been successfully grafted on the surface of magnetic silica microspheres.

Optimization Of Mspe Procedure

Recovery was the best indicator of MSPE method. The recoveries of MC-LR in MSPE process was mainly subjected to several factors including the amount of the MNPs, the extraction time, the pH of sample solution and desorption conditions. In this study, these major factors were investigated using a spiked ultrapure water sample (0.4 µg/L), and all the optimization experiments were conducted three times.

Effect Of The Amount Of Adsorbent

The amount of the adsorbent was investigated so that the adsorbent not only adsorbs sufficient analysts but also remains utilized. 10, 20, 30, 40 and 50 mg of magnetic C_{18} microspheres were discussed and the results were shown in Figure 5A. As shown, the extraction recovery for the analytes increased rapidly when the $Fe_3O_4@SiO_2@C_{18}$ MNPs amount was increased from 10 to 30 mg and then remained almost constant when the amount of the adsorbent was above 30 mg. Based on the above results, the addition of 30 mg $Fe_3O_4@SiO_2@C_{18}$ MNPs was employed for the following studies.

Extracting Time

In the MSPE process, the extraction time is one of the prime factors that influence the extraction of the analytes. The effect of extraction time on the adsorption was investigated from 3-20 min. As shown in Figure 5B, when the sample solution was sonicated for 7 min, the extraction recoveries of MC-LR reached the maximum. This result suggests that the adsorption equilibrium can be achieved at about 7 min. Therefore, an extraction time of 7 min was selected.

Desorption Conditions

The type and volume of elution solvent are vital for the extraction efficiency. So the choice of elution solvent and its optimum volume should be carefully taken into account. Desorption of the analytes from the magnetic adsorbents was studied using 10 mL of

three different solvents including methanol, 99.9% methanol (methanol: TFA (13.07 mol/L) =99.9:0.1, V/V) and 80% methanol (methanol: TFA (13.07 mol/L) =80:20, V/V). The recoveries of MC-LR eluted by methanol, 99.9% methanol and 80% methanol were 68.6%, 72.4% and 58.1%, respectively. The eluting power of 99.9% methanol was the strongest among them. Hence, 99.9% methanol was chosen as elution solvent.

Moreover, the influence of volume of 99.9% methanol was tested. The recoveries of MC-LR eluted by 5, 10 and 15 mL of 99.9% methanol were 56.7%, 72.4%, and 74.1%, respectively. Although the highest recovery was obtained by 15mL of 99.9% methanol elution solvent, the concentration time was longer. Finally, 10 mL of 99.9% methanol was adopted for eluting MC-LR for further work.

In addition, the desorption time was also investigated from 1 to 5 min under sonicating. As shown in Figure 5C, the result indicated that desorption time had obvious effect on the extraction efficiency. The extraction recovery for the analytes increased rapidly when the desorption time was increased from 1 to 3 min and then remained almost constant when the desorption time was 3 min. Thus, the desorption time was selected as 3 min. This result indicated that the desorption process was quick and efficient. However, for the adsorption process, the mass transfer of the analytes from water samples to the solid adsorbent was much slower. So the desorption time was much faster than the extraction time (adsorption, 7min).

Effect Of Solution pH

MC-LR is potentially ionizable compounds. Taking into account the lipophilic phase of the magnetic C_{18} microspheres surface, the neutral (*i.e.*, not ionized) forms of the compounds are expected to be easily extracted. In this sense, pH values ranging from 1 to 6 were studied. As can be seen in Figure 5D, the extraction recovery for MC-LR increased when pH < 4, followed by decreasing when pH > 4. The highest signals were obtained when the samples were prepared at pH 4. The effect of sample pH on the extraction efficiency was consistent with the retention factor *K* of MC-LR on C_{18}.[32] At pH 4, a major portion of the MC-LR was protonated (the neutral form), resulting in stronger adsorption on the surface of the $Fe_3O_4@SiO_2@C_{18}$ MNPs. Therefore, a pH value of 4 was selected for further experiments.

Salt Effect

To investigate the salt effect on the extraction of the MC-LR, NaCl was used to adjust the solution salinity. The results that the peak areas for MC-LR did not obviously increase as the concentration of NaCl increased from 0 to 30%. Therefore, no NaCl was added in the following extractions.

Analytical Performance

Under the optimal conditions mentioned above, the analytical performance of the proposed method was assessed. Six standard solutions with different concentration (0.1,

0.2, 0.5, 1.0, 2.0, 3.0, 5.0, 10.0 μg/L for MC-LR) were obtained by serial dilution with ultrapure water from the standard solution. Working curves were obtained by a least-squares linear regression analysis of the peak area of the analytes versus analyte concentrations. The method presented an excellent linearity in the range of 0.1–10.0 μg/L for MC-LR with the correlation coefficient (r^2) of 0.9996. And the obtained linear regression equation was $y = 120.15\ x – 2.2056$, where y means the peak area, x stands for the concentration of MC-LR. The limit of detection (LOD) calculated by analyzing the spiked sample using a signal-to-noise ratio of 3 was 0.056 μg/L. The limit of quantitation (LOQ) of 0.18 μg/L was achieved for MC-LR. WHO recommended a provisional level of 1 μg/L for MC-LR concentration in drinking water. So, the developed MSPE-HPLC-DAD method proved potentially applicable for MC-LR determination in drinking water samples. On the other hand, the intra-day and inter-day precisions in terms of peak area obtained on the basis of 6 injections were investigated. The relative standard deviations (RSDs) for MC-LR at 0.5 and 5 μg/L based on intra-day precision were less than 5.02% and 3.95%, respectively, while the inter-day remained under 6.15 and 8.22%, respectively. Moreover, a high enrichment factor of 500 was obtained. Therefore, the $Fe_3O_4@SiO_2@C_{18}$ MNPs based MSPE coupled with HPLC-DAD could sensitively and accurately quantify the MC-LR.

Moreover, the reusability of the $Fe_3O_4@SiO_2@C_{18}$ MNPs was examined. In order to investigate the possibility of the reuse, the $Fe_3O_4@SiO_2@C_{18}$ MNPs were reused for

three adsorption-desorption cycles, and nearly constant recovery values were obtained with relative error less than 3%. Also, the FT-IR spectra of the Fe_3O_4@SiO_2@C_{18} MNPs for the first use and after the third use were consistent. So, the results showed the magnetic separation under an external magnetic field could easily reach, and the stable Fe_3O_4@SiO_2@C_{18} MNPs could effectively extract MC-LR at least three repeated cycles without obvious decrease of recovery.

Comparison Of Different Analytical Methods

Table 1 shows the comparison of different analytical methods for the determination of MC-LR. The higher sensitivity achieved is 0.02 μg/L based on an on-line trace enrichment SPE system coupled with LC–DAD.[13] As it is difficult to conduct clean-up in the on-line enrichment system, interference will strongly disturb the accuracy of the results. Although the analysis of MC-LR by HPLC-MS/MS[9,17,18] can provide lower LODs, the instrument is expensive so as to difficultly popularize. The method developed in our current study presented the lower LODs. Excitedly, the method displays excellent reusability and rapid simple magnetic separation, less than 15 min by just using a magnet. On the other hand, compared to previous reports which also employed MSPE,[23,30,33] our method has the advantages of larger sample volume (1 L, most reports using lower volume than 350 mL) and higher enrichment factors (500).

Applications Of The Mspe To Water Samples

In order to evaluate the method applicability, the water sample collected from Qingdao Jihongtan reservoir was analyzed. Before use, the water samples were filtered through 0.45 μm membrane. As seen from Figure 6, no MC-LR was detected in the water sample. The recoveries were obtained by spiked Qingdao Jihongtan reservoir water samples with 0.500, 1.00 and 4.00 μg/L of MC-LR, respectively. The recoveries and RSDs of spiked Jihongtan reservoir water sample were averaged from three replicate runs, as shown in Table 2. The recoveries ranged from 73.3–104%, with RSDs in the range of 2.90–4.30%. In addition, another reservoir water collected in June was studied, possibly eutrophic water sample. Really, as expected, the endogenous MC-LR was detected, 0.51 μg/L. Fortunately, the tested reservoir water sample also satisfied the drinking water quality since the value was lower than the regulated level of 1 μg/L for MC-LR by WHO. Therefore, the developed MSPE using Fe_3O_4@SiO_2@C_{18} MNPs as adsorbents followed by HPLC-DAD proved practically applicable to MC-LR analysis in reservoir water samples.

CONCLUSIONS

In conclusion, a simple, sensitive and robust Fe_3O_4@SiO_2@C_{18} MNPs based MSPE method was successfully developed for the determination of MC-LR in reservoir water samples followed by HPLC-DAD. Good extraction efficiency and high enrichment factor were obtained for MC-LR. The LOD and LOQ were 0.056 μg/L and 0.18 μg/L, respectively. The LOQ was significantly lower than the provisional guideline value

established by the WHO for MC-LR concentrations in drinking water (1.0 μg/L). The pretreatment time was significantly short less than 15 min compared to conventional SPE (2–4 h). And the assay needs no complicated devices. The developed MSPE-HPLC-DAD method provided great potential to analyze practical water samples.

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REFERENCES

1. Duy, T. N.; Lam, P. K. S.; Shaw, G. R. Toxicology and Risk Assessment of Freshwater Cyanobacterial (Blue-Green Algae) Toxins in Water. *Rev. Environ. Contam. Toxicol.* **2000**, *163*, 113–186.

2. Vasconcelos, V. M.; Pereira, E. Cyanobacteria Diversity and Toxicity in a Wastewater Treatment Plant (Portugal). *Water. Res.* **2001**, *35*, 1354–1357.

3. Kunimitsu, K. Chemistry and Toxicology of Cylic Hepatapetide Toxins, the Mi-crocystins from Cyanobacteria. *Microbiol. Cult. Coll*. **1994**, *10*, 5–33.

4. Sivonen, K.; Jones, G. Cyanobacterial Toxins. in: I. Chorus, J. Bartram (Eds.), Toxic Cyanobacteria in Water: a Guide to Their Public Health Consequences, Monitoring and Management. E&FN Spon, London, 1999, Vol. 41.

5. Falconer, I.; Bartram, J.; Chorus, I.; Kuiper-Goodman, T.; Utkilen, H.; Burch, M.; Codd, J. Safe Levels and Safe Practices. in Chorus, I., Bartram, J. (Eds.) Toxic Cyanobacteria in Water–a Guide to Their Public Health Consequences Monitoring and Management. 155-178, on behalf of WHO, London: Spoon Press, **1999**.

6. Chu, F. S.; Huang, X.; Wei, R. D. Enzyme-Linked Immunosorbent Assay for Microcystins in Blue-Green Algal Blooms. *J. Assoc. Off. Anal. Chem.* **1990**, *73*, 451–456.

7. Lawton, L. A.; Edwards, C.; Codd, G. A. Extraction and High-Performance Liquid Chromatographic Method for the Determination of Microcystins in Raw and Treated Waters. *Analyst* **1994**, *119*(7), 1525–1530.

8. Gago-Martínez, A.; Piñeiro, N.; Aguete, E. C.; Vaquero, E.; Nogueiras, M.; Leão, J. M.; Rodríguez-Vázquez, J. A.; Dabek-Zlotorzynska, E. Further Improvements in the Application of High-Performance Liquid Chromatography, Capillary Electrophoresis and Capillary Electrochromatography to the Analysis of Algal Toxins in the Aquatic Environment. *J. Chromatogr. A* **2003**, *992*(1-2), 159–168.

9. Zhang, L. F.; Ping, X. F.; Yang, Z. G. Determination of Microcystin-LR in Surface Water Using High-Performance Liquid Chromatography/Tandem Electrospray Ionization Mass Detector. *Talanta* **2004**, *62*, 193–200.

10. Cong, L. M.; Huang B. F.; Chen, Q.; Lu, B. Y.; Zhang, J.; Ren, Y. P. Determination of Trace Amount of Microcystins in Water Samples Using Liquid Chromatography Coupled with Triple Quadrupole Mass Spectrometry. *Anal. Chimi. Acta* **2006**, *569*, 157–168.

11. Mekebri, A.; Blondina, G. J.; Crane, D. B. Method Validation of Microcystins in Water and Tissue by Enhanced Liquid Chromatography Tandem Mass Spectrometry. *J. Chromatogr. A* **2009**, *1216*, 3147–3155.

12. ISO 20179: **2005** Water Quality-Determination of Microcystins-Method Using Solid Phase Extraction (SPE) and High Performance Liquid Chromatography (HPLC) with Ultraviolet (UV) Detection.

13. Lee, H. S.; Jeong, C. K.; Lee, H. M.; Choi, S. J.; Do, K, S.; Kim, K.; Kim, Y. H. On-Line Trace Enrichment for the Simultaneous Determination of Microcystins in Qqueous Samples Using High-Performance Liquid Chromatography with Diode-Array Detection. *J. Chromatogr. A* **1999**, *848*, 179–184.

14. Ramanan, S.; Tang J.; Velayudhan, A. Isolation and Preparative Purification of Microcystin Variants. *J. Chromatogr. A* **2000**, *883*, 103–112.

15. Ammerman, J. L.; Aldstadt III, J. H. Monolithic Solid-Phase Extraction for the Rapid on-Line Monitoring of Microcystins in Surface Waters. *Microchim. Acta* **2009**, *164*, 185–196.

16. Spoof, L.; Meriluoto, J. Rapid Separation of Microcystins and Nodularin Using a Monolithic Silica C_{18} Column. *J. Chromatogr. A* **2002**, *947*, 237–245.

17. Zhao,Y. Y.; Hrudey, S.; Li, X. F. Determination of Microcystins in Water Using Integrated Solid-Phase Microextraction with Microbore High-Performance Liquid Chromatography–Electrospray Quadruple Time-of-Flight Mass Spectrometry, *J. Chromatogr. Sci.* **2006**, *44*, 359–365.

18. Pavagadhi, S.; Basheer, C.; Balasubramanian, R. Application of Ionic-Liquid Supported Cloud Point Extraction for the Determination of Microcystin-leucine–arginine in Natural Waters. *Anal. Chimi. Acta* **2011**, *686*, 87–92.

19. Man, B. K. W.; Lam, M. H. W.; Lam, P. S.; Wu R. S. S.; Shaw, G. Cloud-Point Extraction and Preconcentration of Cyanobacterial Toxins (Microcystins) from Natural Waters Using a Cationic Surfactant. *Environ. Sci. Technol.* **2002**, *36*(18), 3985–3990.

20. Ma, J. P.; Xiao, R. H.; Li, J. H.; Yu, J. B.; Zhang, Y. Q.; Chen, L. X. Determination of 16 Polycyclic Aromatic Hydrocarbons in Environmental Water Samples by Solid-Phase Extraction Using Multi-Walled Carbon Nanotubes as Adsorbent Coupled with Gas Chromatography–Mass Spectrometry. *J. Chromatogr. A* **2010**, *1217*(34), 5462–5469.

21. Ma, J. P.; Xiao, R. H.; Li, J. H.; Li, J.; Shi, B. Z.; Liang, Y. J.; Lu, W. H.; Chen, L. X. Headspace Solid-Phase Microextraction with on-Fiber Derivatization for the Determination of Aldehydes in Algae by Gas Chromatography–Mass Spectrometry. *J. Sep. Sci*. **2011**, *34*(12), 1477–1483.

22. Alpendurada, M. D. Solid-Phase Microextraction: A Promising Technique for Sample Preparation in Environmental Analysis. *J. Chromatogr. A* **2000**, *889*, 3–14.

23. Song, Y. R.; Zhao, S. L.; Tchounwou, P.; Liu, Y. M. A Nanoparticle-Based Solid-Phase Extraction Method for Liquid Chromatography–Electrospray Ionization-Tandem Mass Spectrometric Analysis. *J. Chromatogr. A* **2007**, *1166*(1-2), 79–84.

24. Liu, Y.; Li, H. F.; Lin J. M. Magnetic Solid-Phase Extraction Based on Octadecyl Functionalization of Monodisperse Magnetic Ferrite Microspheres for the Determination of Polycyclic Aromatic Hydrocarbons in Aqueous Samples Coupled with Gas Chromatography-Mass Spectrometry. *Talanta* **2009**, *77*(3), 1037–1042.

25. Li, J. D.; Zhao, X. L.; Shi, Y. L.; Cai, Y. Q. Mixed Hemimicelles Solid-Phase Extraction Based on Etyltrimethylammonium Bromide-Coated Nano-Magnets Fe_3O_4 for the Determination of Chlorophenols in Environmental Water Samples Coupled with Liquid Chromatography/Spectrophotometry Detection. *J. Chromatogr. A* **2008**, *1180*(1-2), 24–31.

26. Han, Q.; Wang, Z. H.; Xia, J. F. Facile and Tunable Fabrication of Fe_3O_4/Graphene Oxide Nanocomposites and Their Application in the Magnetic Solid-Phase Extraction of Polycyclic Aromatic Hydrocarbons from Environmental Water Samples. *Talanta* **2012**, *101*, 388–395.

27. Zhao, G. Y.; Song, S. J.; Wang, C. Determination of Triazine Herbicides in Environmental Water Samples by High-Performance Liquid Chromatography Using Graphene-Coated Magnetic Nanoparticles as Adsorbent. *Anal. Chim. Acta* **2011**, *78*(1-2), 155–159.

28. Zhang, Z.; Li, J. H.; Fu, J. Q.; Chen, L. X. Fluorescent and Magnetic Dual-Responsive Coreshell Imprinting Microspheres Strategy for Recognition and Detection of Phycocyanin. *RSC Adv.* **2014**, DOI: 10.1039/c4ra00668b.

29. Yu, P. F.; Wang, Q.; Zhang, X. F.; Zhang, X. S.; Shen, S.; Wang, Y. Development of Superparamagnetic High-Magnetization C_{18}-Functionalized Magnetic Silica Nanoparticles as Sorbents for Enrichment and Determination of Methylprednisolone in Rat Plasma by High Performance Liquid Chromatography. *Anal. Chim. Acta* **2010**, *678*(1), 50–55.

30. Sha, Y. F.; Deng, C. H.; Liu, B. Z. Development of C_{18}-Functionalized Magnetic Silica Nanoparticles as Sample Preparation Technique for the Determination of Ergosterol in Cigarettes by Microwave-Assisted Derivatization and Gas Chromatography/Mass Spectrometry. *J. Chromatogr. A* **2008**, *1198–1199*, 27–33.

31. Maddah, B.; Shamsi, J. Extraction and Preconcentration of Trace Amounts of Diazinon and Fenitrothion from Environmental Water by Magnetite Octadecylsilane Nanoparticles. *J. Chromatogr. A* **2012**, *1256*, 40–45.

32. Chen, X. G.; Xiao, B. D; Ao, H. Y.; Chen, X. D.; Xu, X. Q. Relationship between Retention on Chromatography and Charge State of Microcystins. *J. Wuhan Univ. Technol.* **2005**, *27*, 19-21.

33. Ballesteros-Go ´mez, A.; Rubio, S. Hemimicelles of Alkyl Carboxylates Chemisorbed onto Magnetic Nanoparticles: Study and Application to the Extraction of

Carcinogenic Polycyclic Aromatic Hydrocarbons in Environmental Water Samples. *Anal. Chem.* **2009**, *81*, 9012–9020.

TABLE 1. Method Comparisons for Analysis of MC-LR

Methods	Matrix	Adsorbent	LODs (μg/L)	Sample pretreatment time (min)	Ref.
on-line SPE-HPLC/DAD	Surface water	Zorbax CN	0.02	~50	[13]
SPE-LC/MS/MS	Surface water	Waters Oasis HLB	2.6 ng/L	>4 h	[9]
SPME-HPLC/QTOF/MS	Lake-water	CW–TPR fiber	0.8	>45	[17]
CPE[a]-HPLC/MS/MS	Natural waters	Ionic-liquid	0.03	~15	[18]
MSPE-HPLC/DAD	Reservoir water	Fe_3O_4@SiO_2@C_{18} MNPs	0.056	<15	This work

[a]CPE-cloud point extraction

TABLE 2. Determination Results of MC-LR and Method Recoveries in Real Water Samples

Sample	**Spiked (μg/L)**	**Found (μg/L)**	**Recovery (%±RSD, *n* = 3)**
1	0	nd[a]	-
	0.500	0.521	104±2.90
	1.00	0.747	74.7±4.30
	4.00	2.93	73.3±3.50

[a]Not detected.

FIGURE 1. Structure of microcystins.

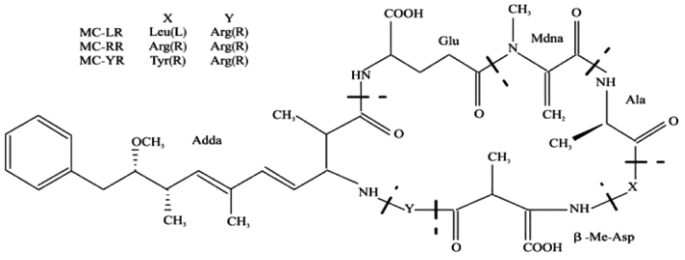

FIGURE 2. Schematic synthesis of C_{18}-functionalized magnetic silica nanoparticles (Fe_3O_4@SiO_2@C_{18} MNPs), and magnetic separation procedure.

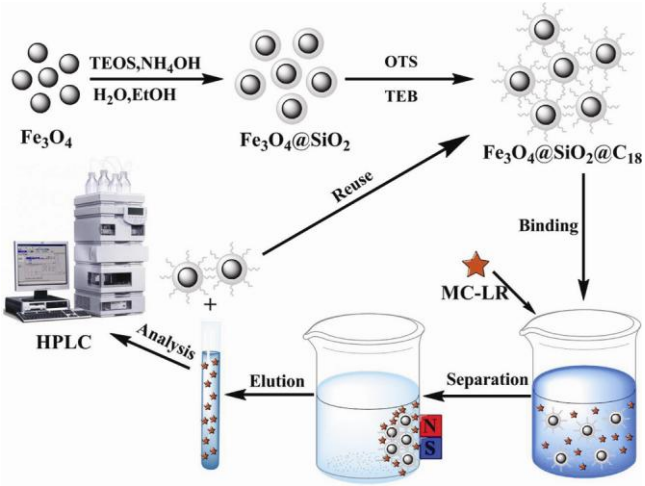

FIGURE 3. The dispersion (left) and separation (right) process; SEM image of the $Fe_3O_4@SiO_2@C_{18}$ MNPs.

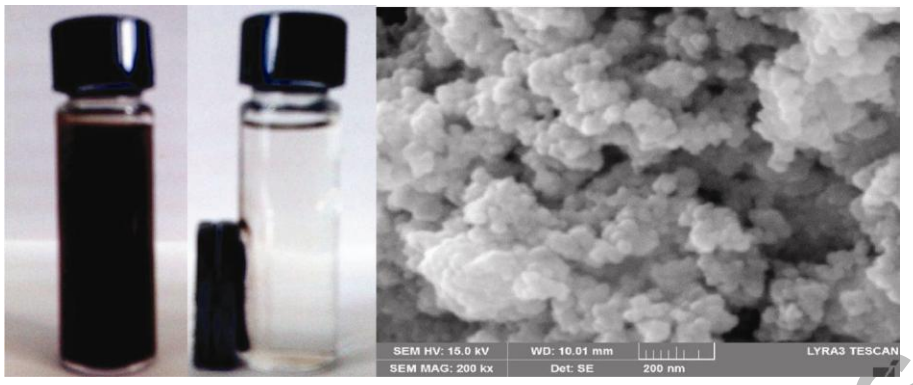

FIGURE 4. FT-IR spectra of (a) Fe_3O_4, (b) $Fe_3O_4@SiO_2$ and (c) $Fe_3O_4@SiO_2@C_{18}$.

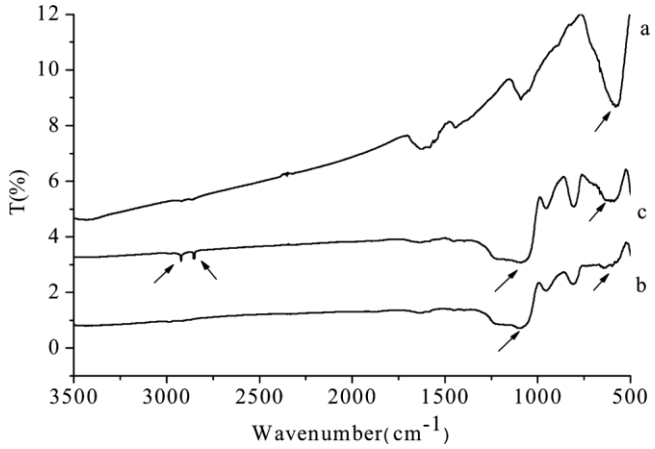

FIGURE 5. Effect of (A) the amount of the magnetic C_{18} microspheres, (B) extraction time, (C) desorption time and (D) solution pH on the extraction efficiency of MNPs-based SPE for MC-LR. Extraction conditions: sample volume, 1.0 L; concentration of MC-LR, 0.4 μg/L; (a) extraction time, 7 min; sample pH, 4; desorption solvent, 2×5 mL of methanol; desorption time, 3 min; (b) the amount of the magnetic C_{18} microspheres, 30 mg; desorption time, 3 min; sample pH, 4; desorption solvent, 2× 5 mL of methanol; (c) the amount of the MNPs, 30 mg; extraction time, 7 min; sample pH, 4; desorption solvent, 2 × 5 mL of methanol; (d) the amount of the MNPs, 30 mg; sample volume, 1 L; extraction time, 7 min; desorption solvent, 2 × 5 mL of methanol; desorption time, 3 min.

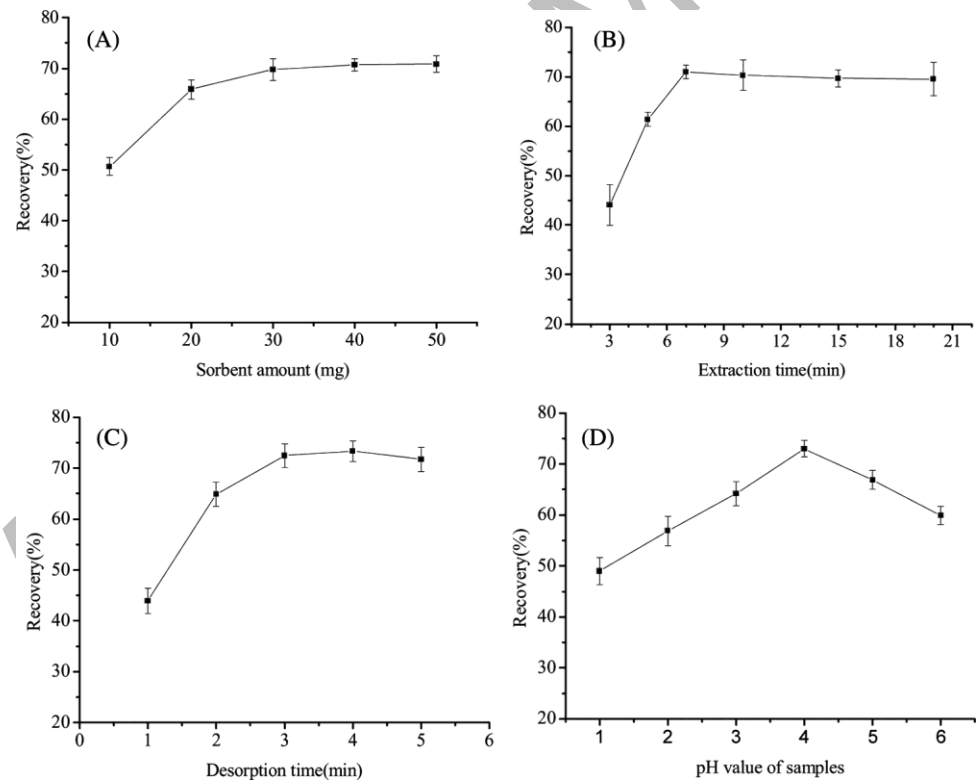

FIGURE 6. HPLC-DAD chromatograms of reservoir water samples (a) with spiking MC-LR after MSPE, (b) without spiking MC-LR after MSPE and (c) no pretreatment and without spiking MC-LR. The spiked concentration of MC-LR standard was 1.0 μg/L. Extraction conditions: sample volume, 1.0 L; the amount of the MNPs, 30 mg; sample volume, 1L; extraction time, 7 min; desorption solvent, 2 × 5 mL of methanol; desorption time, 3 min; sample pH, 4.